Int J Physiol Pathophysiol Pharmacol 2022;14(1):33-47
www.ijppp.org /ISSN:1944-8171/IJPPP0138621

Original Article

$MMEF_{25-75}$ may predict significant BDR and future risk of exacerbations in asthmatic children with normal baseline FEV_1

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Received August 26, 2021; Accepted February 7, 2022; Epub February 15, 2022; Published February 28, 2022

Abstract: (1) Background: Several recent studies on the clinical value of spirometry indexes demonstrated high sensitivity of FEF_{25-75} as a marker of bronchial obstruction in asthmatics with normal baseline spirometry. Our study aims to evaluate the clinical value of maximal mid-expiratory flow in children with asthma. (2) Methods: For two years, 257 children were included - 211 with asthma and 46 healthy controls. Pre- and post-bronchodilator spirometry, atopic status determination and asthma control assessment were performed. (3) Results: The small airway obstruction (SAO) group (FEV_1≥80%, $MMEF_{25/75}$<65%) demonstrated significantly lower values for FEV_1, FEV_1/FVC, PEFR, $MMMF_{25/75}$ and a significant higher bronchodilator response (BDR, ΔFEV_1% init. ≥12%) compared to normal baseline spirometry group (FEV_1>80%, $MMEF_{25/75}$≥65%) (P<0.0001). In addition, we found a statistically significant difference in FEF_{25-75}/FVC median between asthmatics and healthy controls (P<0.0001) regardless of the FEV_1 value. Children with SAO have a 2.338-fold higher risk of poor asthma outcome (OR 95% CI [1.077-5.294]) and a 6.171-fold (OR 95% CI [2.523-15.096]) greater probability of demonstrating positive BDR, compared to children with normal baseline spirometry. $MMEF_{25/75}$ was found to be a good predictor for positive BDR with AUC 0.843 (CI 0.781-0.845) and a best cut-off value of 58.1% (77.8% sensitivity and 78.8% specificity). (4) Conclusion: Our results confirmed that a small but substantial group of asthmatic children with normal baseline FEV_1 and low $MMEF_{25-75}$ are at higher risk for poor asthma outcomes.

Keywords: Childhood asthma, small airway obstruction, spirometry, maximal mid-expiratory flows, asthma control

Introduction

Spirometry is the "gold standard" for diagnosing and monitoring patients with bronchial asthma [1-6]. Small airways are peripheral non-cartilaginous bronchioles with an internal diameter of less than 2 mm, starting from the eighth generation of airways to the periphery of lung parenchyma [7]. Nevertheless, they play a significant role in small airways obstruction, especially in asthmatic children.

As early as the 1990's, it has been demonstrated that patients with asymptomatic asthma showed a more than sevenfold increase in small airway resistance, even though spirometry values (FEV_1, FVC% predicted) and plethysmography resistances were normal [8]. Later, Synek et al. documented the presence of inflammation in both large and small airways [9]. Moreover, persistent uncontrolled inflammation in small airways contributes to poor asthma control and clinical course. A systematic review reported that dysfunction of small airways is associated with poor asthma control, frequent exacerbations, presence of asthma symptoms at night, triggered by allergens and exercise exertion, as well as bronchial hyperreactivity [10]. On the other hand, clinical trials have shown that treatment with small

particle inhaled corticosteroids reduces the number of exacerbations and improves clinical asthma control [11, 12].

According to the ERS/ATS 2005 Taskforce, spirometry results are considered normal at values for FVC≥80% of predicted (or ≥LLN lower limit of normal), FEV_1≥80% from predicted or above LLN and normal FEV_1/FVC ratio [13-19]. Therefore, according to spirometry performance and interpretation, presence of bronchial obstruction is typically characterized by reduced FEV_1 (<80% predicted or below LLN), decreased FEV_1/FVC ratio (Tiffeneau index) and normal FVC (in cases of severe obstruction, it can be even reduced) [18, 19].

One of the limitations of FEV_1 is that it does not adequately reflect the presence of small airway dysfunction because it depends predominantly on FVC in patients with asthma due to increased residual volume [20]. Recent studies have demonstrated the presence of significant small airway dysfunction and obstruction in patients with a normal baseline FEV_1 [18, 21, 22]. Studies in well-controlled asthma indicate the persistence of small airway obstruction and inflammation, regardless of the normal values of the indices reflecting the large airway calibre. Similarly, clinical trials have shown that treatment with small particle inhaled corticosteroids (ICS) reduces the number of exacerbations and improves clinical asthma control [11, 12].

Lung function, evaluated in particular with FEV_1, shows a very poor correlation with the severity of asthma and asthma symptoms, demonstrated in studies in adults and children [23-29]. However, FEV_1 proves to be a good predictor of future risk of asthma exacerbations, and FEV_1/FVC ratio is a more sensitive index in defining the severity of bronchial obstruction [25, 30]. Moreover, FEV_1/FVC along with FEF_{25-75} (FEF_{25-75} - Forced expiratory flow, mid-expiratory phase (at 25-75%) or $MMEF_{25-75}$ - Maximal mid-expiratory flow at 25-75%) are the most commonly reported indices in children that are characterized by preserved FEV_1, regardless of asthma severity [25, 31]. In addition, some studies on FEV_1, FEV_1/FVC and FEF_{25-75} significance define FEF_{25-75} as a more sensitive indicator of bronchial obstruction in both children and adults [18, 32-35]. However, more studies are needed to confirm their significance in asthmatic children.

FEF_{25-75} is a less effort-dependent spirometry index than FEV_1 and is traditionally considered to reflect the calibre of small airways [37, 38]. On the other hand, FEF_{50} and FEF_{25-75} correlate with expiratory lung CT scan data for pulmonary hyperinflation [39-42].

Our study aims to determine and evaluate the clinical significance of peripheral obstruction indices $FEV_{25/75}$, FEV_{50}, FEV_{75}, $MMEF_{25/75}$/FVC, and their post-bronchodilator change in asthmatic children with worsened asthma control but normal FEV_1.

Materials and methods

Study subjects and design

We present a prospective observational study which uses epidemiological, instrumental and immunological methods. For a two-years period (October 2013 to December 2015), we enrolled 257 children divided into two groups - the Asthma group (children with asthma, diagnosed by pediatric pulmonologist), n=211 and Healthy control group, n=46.

Of 211 children with asthma, 77 were girls (36%) and 134 boys (64%), at a mean age of 10.1±3.54 years. In the control group, we enrolled age and sex-matched healthy children without a family history of asthma, a personal history of recurrent bronchial obstruction episodes (not more than two episodes in infancy), and no symptoms of allergic rhinitis enrolled. In addition, asthma patients and control group did not differ statistically by height, weight, and BMI. The asthmatic children were enrolled in the study at their worsening of asthma control (outpatient visits in the Clinic) or asthma symptoms exacerbation (inpatient, 166 children; 78.6%).

Before study enrolment, all parents and children over 12 years old signed written informed consent and child assent, according to the Ethics Committee on Scientific Research requirements at Medical University of Sofia. The study includes epidemiological, instrumental, and laboratory (immunological) methods. All used methods, study design, data acquisition and analysis were performed in compliance with protocols approved by the Ethics Committee on Scientific Research at the Medical University of Sofia (ethical approval No.

5/17.04.2013, scientific project identification code 23D/2013).

Epidemiological methods

We have a detailed history of asthma onset and clinical course of comorbidities and control treatment step for the previous eight weeks for all asthma patients.

The level of asthma control at the last four weeks to prior enrollment was assessed by the validated Bulgarian translation of the Asthma Control Questionnaire (ACQ) for children aged 10-18 years and an interview-based version of the questionnaire for children 6-10 years. For the inpatient group, answers to ACQ refer to the four weeks prior asthma exacerbation for outpatients' group prior to the enrollment visit. ACQ questionnaire and ACQ-IA were provided for use in this study with the express written permission of Prof. Elizabeth Juniper and QOL TECHNOLOGIES Ltd 2003, who owns the copyright for their use [47]. ACQ6 includes six questions reading: night symptoms, severity of the symptoms, the everyday activity limitations, shortness of breath, wheezing and the use of bronchodilator - all in the last seven days before the interview. All of them were answered by the children. In ACQ7, the attending physician adds on the result from FEV_1 before bronchodilator. Each question (including the FEV_1 one) has a 7-point scale (from 0 - no problem/symptoms to 6 - maximal problems/symptom). Each question adds evenly to the final score which is the average number of all - so basically good controlled asthma without symptoms scores 0, loss of asthma control is indicate by score 6.

Asthma severity was determined according to GINA control treatment (Global Initiative for Asthma) step and based on baseline spirometry and extent of asthma symptoms between exacerbations (daytime, nighttime, need for rescue medication, physical activity restriction taken in a 7-degree scale of ACQ) [47, 48].

Instrumental methods - investigation of lung function with spirometry

In 175 of 211 enrolled children with asthma and 46 healthy controls we performed a lung function testing (baseline spirometry and reversibility test - BDRT - bronchodilator responsiveness test) according to ATS/ERS 2005 criteria for quality, repeatability, and reproducibility [13, 15, 16]. In 16 asthmatic children (7.6%), all under the age of 7, the attempt to perform a baseline forced expiratory manoeuvre did not meet quality criteria, another 20 children performed only baseline spirometry without BDRT, and they were excluded from the analysis [12, 13, 15, 16]. The main study groups determination according to recruitment and achievement of technically successful spirometric measurements - baseline and post-BD (BD - bronchodilator) is presented in Figure S1, available in the supplementary file.

All spirometry measurements for the asthmatic group were performed at the Lung function laboratory of the Pediatric Clinic, University Hospital Alexandrovska with Masterscreen Pneumo spirometer '98' (Jager®, Wuerzburg, Germany). The Lung Function laboratory has a child-friendly environment and a specially trained technician (operator) coached and performed all measurements. Spirometry results were presented as a percentage of the predicted value according to the Zapletal reference equation embedded in the Master screen Pneumo software [46]. Maintained and verified barometer and thermometer were used to calculate the BTPS. All quality control and Standard operating procedures were completed according to the approved locally written protocol and ERS/ATS 2005 recommendations [15, 16].

BDRT was performed according to the locally written protocol, following the ERS/ATS 2005 standards and the age of the patients: 15 min after administration of 200 μg (two puffs metered-dose inhaler) Salbutamol (Ventolin) with a spacer or 0.02 ml/kg of the same drug with a nebulizer. In our department, a nebulizer is traditionally used for all inpatients regardless of the severity of asthma exacerbation episode. For outpatients, a metered-dose inhaler was used. Children on bronchodilator therapy were instructed to withhold that medication before baseline testing (at least 4 hours for short-acting β_2-agonist: Salbutamol and 24 hours for long-acting β_2-agonist: formoterol or Salmeterol). BDRT was evaluated by the classical method as a percentage of the change in FEV_1 compared to the baseline measured value and as the absolute change in ml. By ATS/ERS 2005 criteria, BDRT was reported to be significant at $\Delta FEV_1 \geq 12\%$ (percentage of

initial prebronchodilator value, % initial) and/or 200 ml (absolute change (ml) from prebronchodilator value).

Pre- and post-bronchodilator spirometry of children in the control group was performed with a portable Easy One Plus Diagnostic spirometer (ndd Medical Technologies®) connected to a PC, displaying stimulating animation and plotting curves in real-time in an outpatient setting (GPs practice). Individual disposable mouthpiece (spirettes) was used for each child.

Immunological methods

Atopic status assessment was determined by serological examination of total IgE antibodies by ELISA (EUROIMMUN Total IgE ELISA, Medizinische Labordiagnostica AG). The kit uses indirect sandwich ELISA where the microtiter plate is coated with polyclonal anti-human IgE antibodies. Total IgE concentrations in the samples were measured after photometrical evaluation of the optical density of the enzymatic reactions in the wells at 450/630 nm and via a 4-point calibration curve.

To assess the specific IgE antibodies against aero- and nutritional allergens we used semi-quantitative blot immunoassay - Euroline Allergy Profile Pediatrics, Enzyme Allergo Sorbent Test ((Enzyme Allergo Sorbent Test) EAST) with Euroimmune® (Medizinische Labordiagnostica, AG, 2014, Germany). The EUROLINE Pediatric (IgE) test kit includes 28 different respiratory and food allergens: gx (grass mix 2 timothy grass, cultivated rye), t3 (birch), w6 (mugwort), d1 (der. Pteronyssinus), d2 (der. Farina), e1 (cat), e2 (dog), e3 (horse), m2 (Cladosporium her.), m3 (Aspergillus fum.), m6 (Alternaria alt.), f1 (egg white), f75 (egg yolk), f2 (cow's milk), f3 (codfish), f76 (Lactalbumin), f77 (Lactoglobulin), f78 (casein), e204 (bovine serum albumin), f4 (wheat flour), f9 (rice), f14 (soybean), f13 (peanut), f17 (hazelnut), f31 (carrot), f35 (potato), f49 (apple), CCD (CCD marker). The ready test strips were placed on the adhesive foil of the green work protocol prepared beforehand in the EUROLineScan software program, which then calculates the final results of the specific IgE antibodies in patients' samples by evaluating the intensity of the bands in classes from 0 to 6.

Statistical analysis

Statistical analysis of raw data was performed with SPSS®, IBM 2009, version 19 (2010), and Excel (v. 2010). The graphical images presenting the statistics are mainly done using Excel and SPSS v.19. descriptive statistics, Kolmogorov-Smirnoff and Shapiro-Wilks, T-tests, Mann-Whitney, ANOVA - post-hoc-analysis, or Kruskal-Wallis test, respectively, χ^2 or Fisher's Exact test respectively, correlation analysis, and binary logistic regression analysis were used.

ROC curve analysis was also applied where the best cut-off points were chosen those values that are the least distant from the upper left corner of the coordinate system (coordinates 0; 1) or those with the highest sensitivity + specificity (modified Juden index).

The odds ratio (OR) for case-control study was also calculated.

Significance level $\alpha=0.05$ was chosen, i.e., for $p<\alpha$, the null hypothesis was rejected.

Results

Subject's characteristics

Of 211 children in the Asthma group, 77 were girls (36%) and 134 boys (64%), at a mean age of 10.1±3.54 years- or girls 10.5±3.75 years and boys 9.9±3.41 years. Children were predominantly 7 to 15 years of age, 12.9% of children were preschoolers, and 8% were over 15. The main epidemiological characteristics of asthmatic children are presented in **Table 1**.

In the healthy control group age and sex-matched healthy children without family history of asthma, without personal history of recurrent bronchial obstruction episodes (not more than two episodes in infancy) and no symptoms of allergic rhinitis were enrolled. The asthma patients and control group did not differ statistically by height, weight, and BMI (Table S1, available in supplementary file).

In the study population, asthmatic children reported an average of 2 exacerbations of asthma in the previous 12 months and an average of one hospitalization and/or need of systemic corticosteroid for more than 3 days. For the previous 12 months, children lost an average

Table 1. Subject's characteristics in the Control group and asthma group

Parameter	Control group (N=46)	Asthma group (N=211)	p
Birth sex, male	28 (60.9%)	134 (63.5%)	n.s.
Birth sex, female	18 (39.1)	77 (36.5%)	n.s.
Age, years, mean (SD)	10.26 (2.98)	10.18 (3.54)	n.s.
Hight, cm, mean (SD)	144.75 (16.987)	143.33 (18.660)	n.s.
Weight, kg, mean (SD)	37.75 (13.645)	40.61 (16.661)	n.s.
BMI, mean (SD)	17.34091 (3.90)	18.87008 (3.00)	n.s.

Table 2. Indicators of baseline spirometry and bronchodilator response in control and asthma group

Parameter (median)	Control group (N=46)	Asthma group (N=195)	p
FVC	99.8%	91.8%	<0.0001
FEV_1	98.5%	85.4%	<0.0001
FEV_1/FVC	91.5%	92.1%	<0.0001
PEFR	91.0%	81.7%	<0.0001
$MMEF_{25/75}$	99.5%	52.3%	<0.0001
D FEV_1% init.*	3.0%	14.50%	<0.0001
D FEV_1 abs., l*	0.050	0.216	0.004

*asthma BDR group (n=175).

Table 3. Distribution of patients (total, girls, boys) according to ACQ6 and GINA asthma control

Asthma control	total N (%)	boys N (%)	girls N (%)	p
ACQ6 score				n.s.
Under 0.75	39 (35.8%)	27 (38.6%)	12 (30.8%)	
0.75-1.5	16 (14.7%)	6 (14.3%)	10 (15.4%)	
Above 1.5	54 (49.5%)	33 (47.1%)	21 (53.8%)	
GINA score				n.s.
No one	29 (26.6%)	11(28.2%)	18 (25.7%)	
1-2	19 (23.9%)	7 (17.9%)	26 (27.1%)	
3-4	33 (49.5%)	21 (53.8%)	54 (47.1%)	

of four days of school due to asthma control deterioration. Limitation of physical activity was reported by 28.3%, exercise-induced attacks - 22.3%, and allergen-induced attacks - 19.5% of children (**Table 1**).

Family history of bronchial asthma was reported in almost half of children - 48.4% (n=103), with 25% (n=53) of children in their immediate family members (mother, father, brothers, or sisters). In 16 children, there was evidence of bronchial asthma in more than one family member.

In 47.9% (n=101) of the children, there was personal history data for atopy (food and/or drug allergy, atopic dermatitis, followed by allergic conjunctivitis, insect allergy, cow's milk allergy, urticaria with an unspecified causative agent, etc.).

Indicators of baseline spirometry and bronchodilator response differed significantly between children with asthma and healthy controls (**Table 2**) as well as between children with asthma grouped according to the degree of impaired baseline FEV_1 (>80%, <80%≥70%, <70%≥60% and <60%). A significant difference in BMI was found in the four groups according to baseline FEV_1. Children with the worst baseline spirometry - lowest FEV_1<60% had a higher BMI than children with mild bronchial obstruction (P=0.045).

Asthma control as determined by ACQ6 or GINA score does not differ statistically between boys and girls (**Table 3**).

Concomitant allergic rhinitis, diagnosed by specialist (seasonal/year-round), is present in 54.9% (n=116) of children (59% of boys and 48% of girls), more often in school-age children (P=0.003).

One hundred twenty-four children (59%) were CS naïve. They had not received systemic control treatment in the previous eight weeks. Of these, 45 (36%) was children with newly diagnosed bronchial asthma - they were treated with short-acting beta-agonist (Salbutamol) with/without systemic CS in more severe wheezing episodes prior the asthma diagnosis. The remaining 78 (64%) of the CS naïve children are on ICS *when needed* regimen, or were enrolled in the study during the control treatment break for the summer period. Of the 87 children (41%) with systemic control treatment, 21 (24%) were on monotherapy with leukotriene receptor antagonists (LTRA), 42 (48%) were on ICS (Budesonide or Fluticasone propionate), 10 (12%) were on a combination of ICS with LTRA, 11 (13%) on

combined inhaler (ICS with LABA - Budesonide/Formoterol or Fluticasone Propionate/Salmeterol) and three children (3%) on combined inhaler plus LTRA. There was no statistically significant difference in the control treatment between girls and boys.

We found a significant discrepancy in the classification of asthmatic children by severity of the asthma using the three methods (baseline FEV_1 (spirometry), control treatment step and clinical control (ACQ)). The severity of asthma, defined by the control treatment and based on symptoms, was significantly lower than that based on spirometry results (Table S2, available in supplementary file).

Baseline spirometry indices

Baseline spirometry indices were conventionally divided into two groups - those reflecting calibre/function predominantly of large airways, namely FEV_1 and PEFR, and those reflecting calibre/function predominantly of small airways, respectively - $MMEF_{25/75}$ and $MMEF_{75}$. We found that FEV_1/FVC ratio correlated better with the indices reflecting the small airways ($P<0.001$) at Spearman's rho =0.746 for $MMEF_{25/75}$; 0.680 for $MMEF_{75}$ and 0.848 for $MMEF_{25/75}$/FVC, while for PEFR the correlation coefficient was 0.438 and for FEV_1 0.571, respectively.

We also found that indexes FEV_1 and PEFR reflect large airway calibre in contrast with the $MMEF_{25/75}$, $MMEF_{75}$, $MMEF_{50}$, FEV_1/FVC which predominantly reflect small airways. The mean BDR (% change) of the FEV_1 is 17.78% (≥13,9%), PEFR - 16.06% (≥17,69), FVC - 8.1% (≥12.9%) and for the $MMEF_{25/75}$ - 57.65 (≥76,5).

According to baseline spirometry, we divided asthmatic children into two main groups: children with normal lung function ("normal" FEV_1≥80%) and impaired lung function ("abnormal" FEV_1<80%).

Children with normal FEV_1≥80% were divided into 4 subgroups, depending on baseline FEV_1/FVC and baseline $MMEF_{25/75}$: group A - children with "normal function" - (with normal FEV_1/FVC≥85%, normal $MMEF_{25/75}$≥65%), group B - children with "low Tiffeneau" (FEV_1/FVC<85%, $MMEF_{25/75}$≥65%), group D - children with "low Tiffeneau and abnormal $MMEF_{25/75}$" (FEV_1/FVC<85%, $MMEF_{25/75}$<65%), group C - children with isolated "low $MMEF_{25/75}$" (FEV_1/FVC≥85%, $MMEF_{25/75}$<65%) [17] (Figure S2, available in supplementary file).

The group of children with Small Airways Obstruction (SAO) is defined as the cases with normal baseline FEV_1 (≥80%) and $MMEF_{25-75}$ (<65%), regardless of baseline Tiffeneau index (FEV_1/FVC).

In patients with bronchial obstruction (FEV_1< 80%), with an increase in obstruction and the FEV_1% predicted reduction, FEV_1/FVC, PEFR and $MMEF_{25/75}$ decreased proportionally (**Figure 1**).

Small airway obstruction - clinical value of the baseline maximal mid-expiratory flows - $MMEF_{25/75}$, $MMEF_{50}$, $MMEF_{75}$

When grouping children with normal baseline FEV_1≥80% in three groups (group A, B, D in Figure S2, available in supplementary file), the presence of peripheral obstruction was found to increase the likelihood of exercise attacks 5.08 times (OR 5.079, 95% CI 1.461-17.653). No significant difference (OR; odds ratio; case-control study) was found in testing the other risks (hospitalization, exacerbation, physical activity limitation, allergic contact attacks), as well as the risk domain.

Adding a fourth group "C" - children with an isolated "low $MMEF_{25/75}$" and normal Tiffeneau index, we found that hospitalization was a protective factor for "normal" baseline lung function (OR 0.449, 95% CI 0.206-0.978).

Children with peripheral obstruction, a so-called SAO group (FEV_1≥80%, $MMEF_{25/75}$< 65%), had significantly lower FEV_1, FEV_1/FVC, PEFR, $MMME_{25/75}$, and significantly higher BDR (ΔFEV_1% initial and $\Delta MMEF_{75/25}$% init.) compared to those with normal function (FEV_1>80%, $MMEF_{25/75}$≥65%) ($P<0.0001$). There was no significant difference in morbidity (risk and risk domain) - hospitalizations, exacerbations in the previous year, exercise and allergen induced exacerbations, restriction in physical activity, BMI difference and presence of allergic rhinitis between the two groups (Table S3, available in supplementary file).

We compared the distribution of asthmatic children with reduced $MMEF_{25-75}$ (<65%) in the

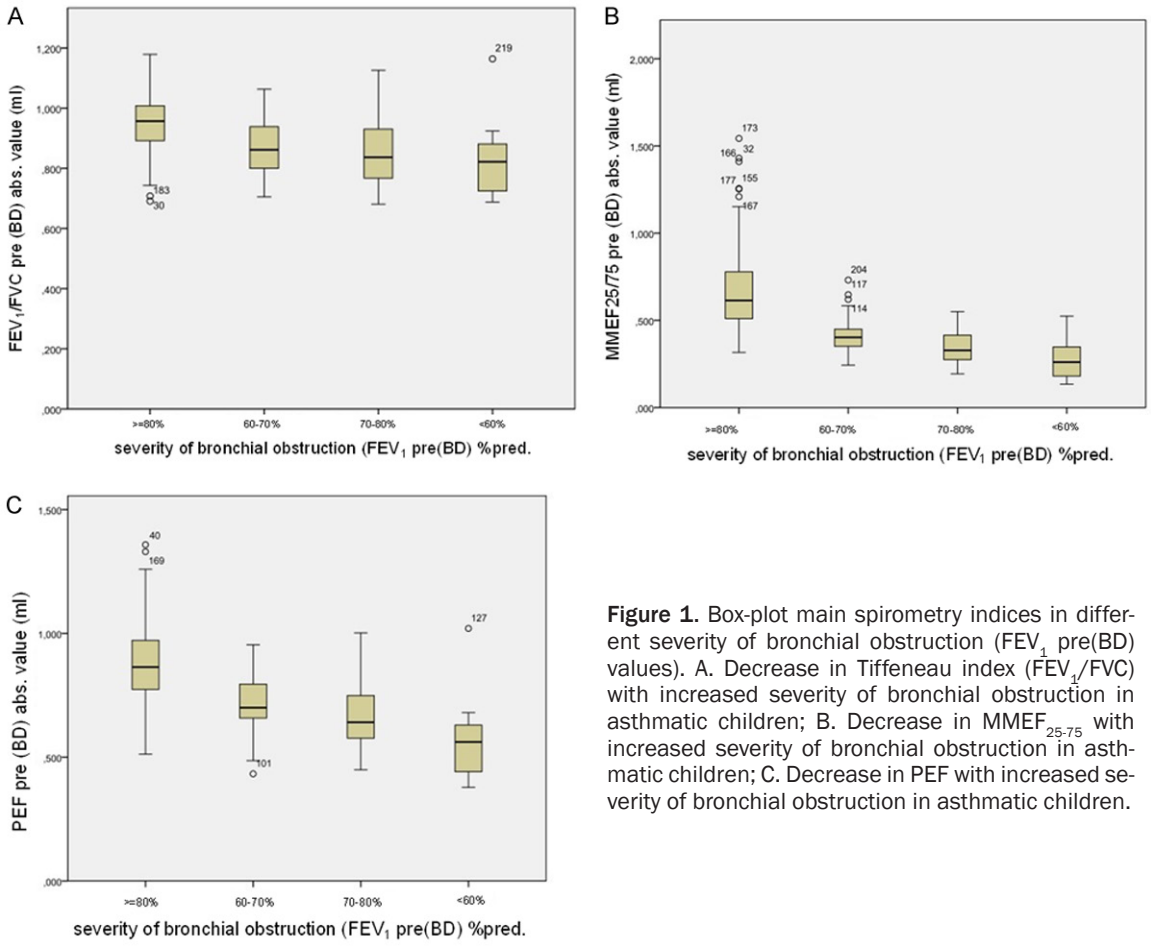

Figure 1. Box-plot main spirometry indices in different severity of bronchial obstruction (FEV_1 pre(BD) values). A. Decrease in Tiffeneau index (FEV_1/FVC) with increased severity of bronchial obstruction in asthmatic children; B. Decrease in $MMEF_{25-75}$ with increased severity of bronchial obstruction in asthmatic children; C. Decrease in PEF with increased severity of bronchial obstruction in asthmatic children.

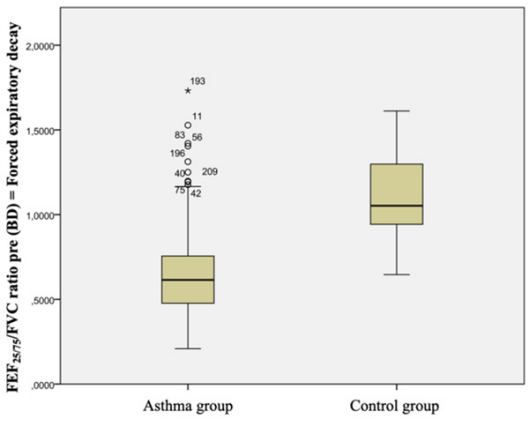

Figure 2. Box-plot index $MMEF_{25-75}$/FVC (Forced expiratory decay) in healthy controls and children with asthma (median, IQR).

groups of children with normal and those with reduced FEV_1. A significant difference was found between both groups - 55.9% of children with normal FEV_1 and 98.5% of children with reduced FEV_1 had peripheral (small airways) obstruction ($MMEF_{25-75}$<65%) (Table S4, available in supplementary file).

Children with small airway obstruction (SAO, FEV_1≥80%, and $MMEF_{25/75}$<65%) have been found to possess a 2.338-fold higher risk of development of one of the risk domain elements (OR 95% CI 1.077-5.294). Children in this group had 3.736-fold (OR 95% CI 1.007-13.860) higher risk to demonstrate a decrease in FEV_1/FVC<85% and 3.857-fold (OR 95% CI 1.518-9.801) higher probability of a low PEF<80%. It was also found that this group of children had a 5.9-fold (OR 95% CI 2.487-13.998) greater probability to demonstrate positive BDR for $MMEF_{25/75}$ and 6.171-fold (OR 95% CI 2.523-15.096) - for FEV_1 over 12%, compared to children with normal baseline FEV_1≥80%, without small airway obstruction ($MMEF_{25-75}$≥65%).

Analyzed with binary logistic regression, the results showed a similar trend. It was confirm-

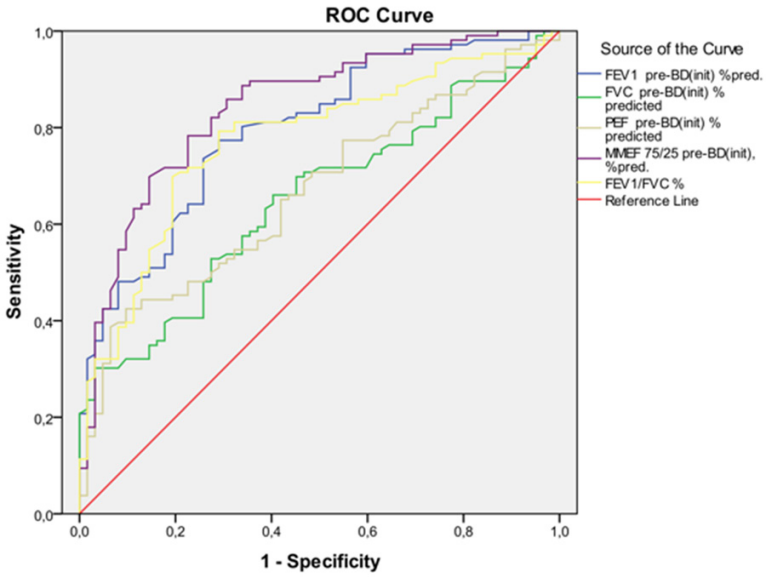

Figure 3. ROC curve for the predictive value of baseline spirometry to reveal a positive BDRT (ΔFEV_1% init. ≥12%).

ed that at $MMEF_{25-75}$<65% (peripheral obstruction regardless of baseline FEV_1), the risk of occurrence of a risk domain element was increased 2.27 times (HR 95% CI 1.120-4.603) (Table S5, available in supplementary file).

Among asthmatic patients with "normal" lung function (FEV_1≥80%) we compared asthma control (ACQ6 and ACQ7 score) in the subgroups of children with and without peripheral obstruction (normal and low $MMEF_{25-75}$). The results indicated no difference in asthma control according to the ACQ6/ACQ7 score between the two groups (borderline significance, p=0.051). This may be due to the small sample size (low number of patients with normal or low $MMEF_{25-75}$ in the subgroups with normal baseline spirometry, according to pre-FEV_1). There was a tendency for children with peripheral obstruction to have poor asthma control.

We defined the so-called "risk domain" - number of hospitalizations for the previous year (≥1), exercise-induced exacerbation (≥1) for the previous year, history of allergen-induced exacerbation, school absenteeism due to asthma in the last 12 months (≥5), lack of asthma control (ACQ score ≥1.5), atopy (elevated total IgE titer according to the age and/or positive specific IgE), concomitant obesity and/or allergic rhinitis (AR), diagnosed by otorhinolaryngologic, very low FEV_1<60%.

Flow volume loop's shape - clinical value of the baseline and post-bronchodilator forced expiratory decay ($MMEF_{25-75}$/FVC index)

We calculated the pre- and post-bronchodilator $MMEF_{25-75}$/FVC index (Forced expiratory decay, informative of the flow volume loop's shape) in the group of children with asthma and in the healthy controls group. At a ratio approximately equal to 1.0, the shape of the loop was linear (normal), and in the ratio <1 the shape was obstructive (concave). There was a statistically significant difference between the median $MMEF_{25-75}$/FVC of healthy children and those with asthma (P<0.0001). In children with asthma, the curve was markedly obstructive regardless of the value of FEV_1 (Table S6, available in supplementary file and **Figure 2**).

There was a significant difference in the pre- and post-bronchodilator shape of the flow-volume loop ($MMEF_{25-75}$/FVC value) in the group of children with asthma (P≤0.0001). Even post-bronchodilator, the shape of the curve in children with asthma retains its markedly obstructive character ($MMEF_{25-75}$/FVC is below 1.0-0.75, IQR 0.57-0.91) (Table S7, available in supplementary file).

Evaluation of the $MMEF_{25/75}$ as a predictive index for positive BDR

ROC curve method was used to evaluate sensitivity and specificity of baseline spirometry predictors for a positive BDR (standard criteria ≥FEV_1% init. ≥12%). $MMEF_{25/75}$ was found to be the strongest predictive index with an area under the curve 0.843 AUC (CI 0.781-0.845). In both of the best cut-off values for $MMEF_{25-75}$, combining the best sensitivity and specificity (77.8% or 78.8%) for the specific patient population, was determined 58.1%, which is close to that reported in the literature (60%, 65%). A threshold for $MMEF_{25/75}$<65% shows a high sensitivity of 82.7% but a lower specificity of 54.5%.

The AUC for FEV_1% pred. was 0.796 (95% CI 0.728-0.863, P<0.001), for FVC% pred. was estimated as 0.650 (95% CI 0.568-0.733, P=0.001), for PEF% pred. - 0.666 (95% CI 0.585-0.748, P<0.001), $MMEF_{75/25}$% pred. - 0.843 (95% CI 0.781-0.905, P<0.001), and for FEV_1/FVC% - 0.773 (95% CI 0.701-0.845, P<0.001). The data are presented on **Figure 3**.

Discussion

We confirmed that pulmonary function, in particular presented as FEV_1% pred., showed very poor correlation with asthma severity and symptoms, which has been demonstrated in several studies in both adults and children [24-26, 27, 49, 50]. However, baseline FEV_1% pred. is proving to be a good predictor of the future risk of exacerbations [30]. The FEV_1/FVC ratio (Tiffeneau index) is more sensitive when defining the severity of bronchial obstruction [25]. In addition, FEV_1/FVC, along with FEF_{25-75}, are the most commonly affected indicators of childhood asthma characterized by stored FEV_1, regardless of the severity of the disease [25, 51].

We agree with the published strong evidence that underestimating the severity of asthma leads to suboptimal treatment and impaired quality of life [52, 53]. For the purpose of the study, we defined the severity of asthma according to three criteria: according to the stage of GINA control treatment, according to the baseline spirometry, and according to the degree of asthma symptoms outside the attack (daytime, nighttime, use of rescue medication, restriction in physical activity taken from the 7-point ACQ scale). We found a significant discrepancy in the classification of children by severity using the three methods. Asthma severity, defined by the stage of control treatment and based on symptoms (ACQ and GINA score), is significantly lower than that based on spirometry results. Our results are consistent with the literature data on the lack of association between symptom severity, medication intensity, and FEV_1% of predicted values [25]. Nair et al. also demonstrate that the use of spirometry identifies a large percentage of children with abnormal pulmonary function who have been evaluated as having mild asthma according to history and physical examination [54]. Schifano et al. investigate the concordance between spirometry and asthma symptoms when assessing asthma severity and initiating control treatment in children. The results of their study show that in 36% of the children tested, the severity of asthma determined by the basis of symptoms, was lower than that based on the results of spirometry [26]. Ming-Sheng Lee et al. also found a weak correlation between lung function and the level of symptoms assessed by ACT/C-ACT (asthma control test, childhood asthma control test) [55]. According to the authors, pulmonary function and symptoms at the age of 5-11 years have different implications for asthma control, with Lung function tests (LFT) reflecting the condition of the respiratory tract on the day of the study, whereas asthma control questionnaires (ACQ/ACT) provide information about the patient's symptoms for the previous month/week. Our results also confirm that the combination of LFT and ACT/ACQ allows the detection of more patients with inadequate asthma control, therefore we strongly advocate this method to be included in everyday practice of pediatric pulmonologists.

It was noted that more than half of studied children (65%, n=124) showed a normal baseline $FEV_1 \geq 80\%$ during exacerbation or poor asthma control. Our results confirmed the results of a number of studies in children, in which baseline FEV_1 and spirometry in general show low sensitivity in detection of bronchial obstruction, and 80% of asthmatic attacks occur in children with normal FEV_1 [56]. Restriction on use of FEV_1 alone in pediatric asthma is demonstrated and by Bacharier et al., with a lack of association between symptom severity, drug intensity, and FEV_1% of predicted value [25]. Consistent with literature, our results suggest that making a clinical diagnosis based on a single measured value of baseline FEV_1 may underestimate the diagnosis, severity, and choice of control treatment [56, 57].

When dividing baseline spirometry indices from those reflecting caliber/function mainly of large airways (FEV_1 and PEFR - Peak expiratory flow rate) and those reflecting caliber/function mainly of small airways ($MMEF_{25/75}$ and $MMEF_{75}$), we found a stronger correlation of the Tiffeneau index (FEV_1/FVC) with indicators reflecting small airways. Francisco et al. demonstrate that indicators reflecting the caliber of small airways (FEF_{25-75}, FEF_{50}, FEF_{75}) in child-

hood are more sensitive in detecting bronchial obstruction than those reflecting large airways (FEV_1 and PEFR), and FEV_1/FVC correlates better with small lung volumes [58]. Similar to our observations, Vilozni et al. found low sensitivity of the most commonly used parameters (FEV_1, $FEV_{0.5}$, FVC and PEFR) in the detection of bronchial obstruction compared to mean $FEF_{25/75}$% and FEF_{50}% flow rates [59].

We found that children with peripheral obstruction (SAO, FEV_1>80%, and $MMEF_{25/75}$<65%, regardless of baseline FEV_1/FVC) had a 2.388-fold greater risk of developing any of the risk domain elements (OR 95% CI 1.077-5.294). The same result was obtained with the logistic regression method (HR 2.27 95% CI 1.120-4.603). Children in this group are 5.9 times (OR 95% CI 2.487-13.998) more likely to be positive BDR for $MMEF_{25/75}$ and target 6.171 times (OR 95% CI 2.523-15.096) for ΔFEV_1 above 12% compared to children with normal baseline FEV_1≥80% without peripheral obstruction ($MMEF_{25-75}$≥65%). We have also shown a tendency of poor asthma control in children with peripheral obstruction.

Several studies have linked small airways function to asthma symptoms [60]. Recently, Schiphof-Godart et al. selected patients with SAO based on FEF_{50} from spirometry and R5-R20 from IOS and found that patients with SAO have more frequent symptoms induced by physical exertion, allergen contact and climate change [61]. Sirux V et al. found that reduced baseline FEF_{25-75} levels increased the risk of long-term asthma persistence and more severe BHR (bronchial hyperreactivity), regardless of FEV_1 levels, i.e., regardless of the effect of large airways [62]. According to Kanchongkittiphon et al. results, 79% of children with persistent asthma have normal FEV_1≥80% and 63% normal FEV_1/FVC≥80%, and a low FEF_{25-75} provides additional information on asthma control regardless of FEV_1 and FEV_1/FVC [63]. Using logistic regression, Gibb et al. found that FEF_{25-75}<60% was associated with a 2.50-fold higher likelihood of hospitalization in the previous year (OR 2.50, CI 1.17-5.35) than FEF_{25-75}≥60%, again regardless of baseline FEV_1 [64].

Tosca et al. demonstrate a correlation between FEF_{25-75}, sIgE for house dust, and FeNO in children with AP and/or asthma and suggest a likely direct link between the markers of allergic inflammation and SAO [65]. The inversely proportional relationship between FEF_{25-75} (≤65%) and FeNO in children with asthma, as well as the significance of FEF_{25-75} as an indirect marker of inflammation in airways has been confirmed by other authors [66-68]. Similar to the results of Tosca et al., we found a significantly higher percentage of children sensitized to microarrays in house dust (D. farinae and D. pteronyssimus) in the peripheral obstruction (SAO) group compared to normal function children, P=0.037.

The MFVL (Maximal flow volume loop) evaluation of spirometry interpretation process also includes visual inspection of the shape of the curve. An objective indicator providing information on the form of an MPC is the FEF_{25-75}/FVC ratio (a surrogate marker for the ratio of airway size to lung size) [58, 59, 69, 70]. Vilosny et al. examined the form of MFVL (FEF_{25-75}/FVC) and found that the contour of the curve in children differs significantly from that in adults and may appear "normal" in children with mild obstruction [59]. Our results showed a statistically significant difference between the $MMEF_{25-75}$/FVC index of healthy children and children with asthma (P<0.0001). As expected in patients with asthma, the curve is markedly obstructive, regardless of the baseline FEV_1 value. A significant difference in MFVL shape ($MMEF_{25-75}$/FVC) was also found in the asthma group in response to administration of a bronchodilator (P<0.0001), which, however, maintained its markedly obstructive nature ($MMEF_{25-75}$/FVC below 0.75 IQR [0.57-0.91]).

A significant difference was observed in mean values of baseline spirometry, BDR (ΔFEV_1 and $\Delta MMEF_{25-75}$) and $MMEF_{25-75}$/FVC in the group of peripheral obstruction (SAO) and normal function children. Children with SAO had significantly lower values of all studied indices and higher BDR (P<0.0001).

There is a great interest in BDR in children with normal baseline spirometry. A cut-off between 60% and 70% for FEF_{25-75} is best in predicting positive BDR (ΔFEV_1≥12%) with normal baseline FEV_1≥80% [71]. According to Simon et al. FEF_{25-75}<68% showed 95% sensitivity and 63% specificity for predicting a 20% increase in FEV_1 after Albuterol inhalation [31]. In our sensitivity and specificity analysis of the main indicators

of baseline spirometry for prediction of positive BDR by classical criteria (ΔFEV_1% init. ≥12%) we obtained similar results. The highest diagnostic power (AUC 0.843 CI 0.781-0.845) demonstrated $MMEF_{25/75}$ with the best cut-off combining maximum sensitivity and specificity (77.8%, or 78.8%) below 58.1%, which is close to the lower limit of reference value for this indicator reported by literature data (60%, 65%) [32, 72]. $MMEF_{25/75}$ threshold <65% in the study population showed a high sensitivity of 82.7% but a lower specificity of 54.5%.

Assessment of small airways condition using spirometry has its advantages and disadvantages over the gold standard IOS and requires careful attention in the interpretation process. Use of FEF_{25-75}% in adults is not recommended because of high indicator variability in healthy subjects, whereas in childhood it is more widely used. FEF_{25-75}% is an indicator of high physiological sensitivity for predicting bronchial reversibility [32]. In childhood, FEF_{25-75}% provides additional information on clinical status and inflammation of PD, correlates well with BD in patients with normal baseline FEV_1 and is associated with morbidity and severity of pediatric asthma [71].

In addition to its high variability, another drawback of the FEF_{25-75} indicator is the lack of consensus on its normal value [73]. Ciprandi et al. propose a threshold for "normal" FEF_{25-75}, showing that 45% of children with mild asthma have a value for FEF_{25-75} below 65% of what is predicted. Simon et al. in the CAREN (Childhood Asthma Research and Education Network), using the ROC method, find that a threshold of 68% for FEF_{25-75} may predict positive BDR (20% change in FEV_1) in patients with mild asthma and normal FEV_1 [32]. Mandadzhieva et al. found that healthy children exposed to secondhand smoke had lower values for FEF_{50} and FEF_{75}, suggesting that initial chronic inflammation with this localization would lead to peripheral obstruction [74].

According to a recent large multinational and multicenter study by Quanjer et al., values of FEF_{25-75}% and FEF_{75}% below the LNN (<-1.645 z-score, according to the GLI 2012 reference equation) are only found in 2.75% and 1.29% respectively, with FEV_1, FVC and FEV_1/FVC within the reference range (above LLN). According to the same study, FEF_{25-75}% miss bronchial obstruction in 2.9% of cases and FEF_{75}% in 12.3% of cases. The authors conclude that indicators reflecting maximum flow rates in the middle part of the expiratory flow-volume loop do not provide additional information to those of FEV_1, FVC, and FEV_1/FVC when making clinical decisions [75].

In our real-life study, the analyzed values of spirometry indices are calculated as percentages of the predicted Zapletal reference equation, which is traditionally used in the Bulgarian population of children. The use of the GLI 2012 reference equation and the z-score method in the interpretation of spirometry has not yet been widely adopted and accepted in the daily practice of pediatric pulmonologists in Bulgaria. When working with older reference equations, and especially when using a fixed cut-off for LNN, the FEF_{25-75}% indicator provides valuable clinical information in children with asthma and normal baseline FEV_1.

Conclusions

Based on literature references and the results we have obtained, we can conclude that there is a small but essential group of asthmatic children with a normal baseline FEV_1 and an abnormal $MMEF_{25-75}$. Children in this group are at an increased risk of adverse outcome (exacerbations, hospitalizations, progressive reduction of pulmonary function, persistent BDR) and may need to undergo a higher step of control treatment after careful assessment of adherence to the therapeutic plan and evaluation of inhaler technique.

In children with asthma and normal baseline FEV_1 and Tiffeneau index (<LLN), $MMEF_{25-75}$ may be considered a marker that predicts BDR positivity (delta FEV_1% init.), asthma control severity, and risk of exacerbations, physical activity attacks, both in scientific research and in clinical practice. However, average debits and their formal inclusion in official guidelines remain limited due to their high variability. Baseline spirometry and Asthma Control Assessment Questionnaire (ACQ) correlate poorly, but administered in combination may better identify children at risk for loss of control, exacerbation, and progressive pulmonary function impairment.

Acknowledgements

We would like to thank Tea Alexandrova, UMHATEM "N. I. Pirogov" Pediatric department for providing technical support in editing the final English version of the manuscript. This work was supported by a grant from the Medical University of Sofia (Council of Medical Science, project no. 35D/2013, grant no. 23D/2013).

Disclosure of conflict of interest

None.

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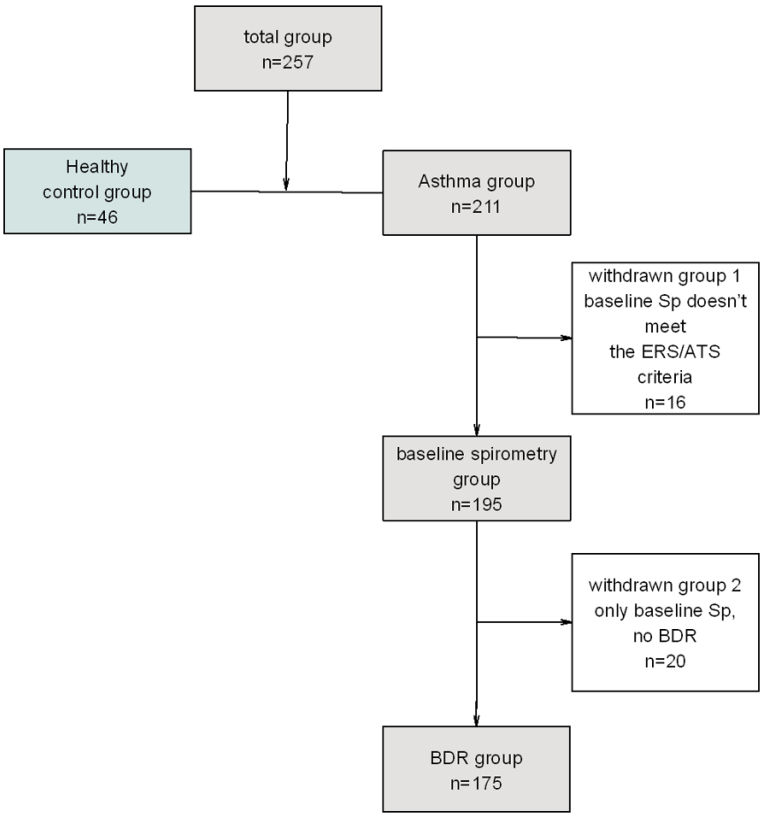

Figure S1. Flow chart showing recruitment and achievement of technically successful spirometric measurements - baseline and post-BD (BD - bronchodilator).

Table S1. Basic characteristics of the asthmatic children included in the study. ACQ (n=109), baseline spirometry (n=195), BDRT (n=175), total IgE (n=85), EUROIMMUN Ped (n=109)

Parameter	Total	Boys	Girls	p*
Mean age, years ± SD	10.1±3.54	10.5±3.75	9.9±3.41	n.s.
Family history - asthma, %	102 (50%)	36 (47.4%)	66 (51.6%)	n.s.
Family history - atopy, %	106 (52%)	41 (53.9%)	64 (50%)	n.s.
Parent - smoker, %	88 (75.9%)	58 (77.3%)	30 (73.2%)	n.s.
Mother - smoker, %	69 (60%)	22 (55%)	47 (62.7%)	n.s
History of atopy, %	101 (49%)	38 (50.7%)	63 (48.1%)	n.s.
Allergic rhinitis, %	112 (54.4%)	35 (46.7%)	77 (58.8%)	n.s.
"Wheezing" in infancy, n, %	102 (51.5%)	73 (57.5%)	29 (40.8%)	0.006
Exacerbations (median, IQR)#	2 (1-3.5)	2.3±2.3	3±2	n.s.
Hospitalizations (median, IQR)#	0 (0-1)	1.0±1.14	1.12±1.15	n.s.
History for exercise induced exacerbation#	39 (22.3%)	15 (25.4%)	24 (20.7%)	n.s.
History for allergen induced exacerbation#	34 (19.5%)	9 (15.3%)	25 (21.7%)	n.s.
Physical activity limitation#	49 (28.3%)	18 (30.5%)	31 (27.2%)	n.s.
School absence, days (median, IQR)#	4 (0-7)	5 (0-8)	6 (0-6)	n.s.
FEV_1% pred. (median, IQR)	85.4 (75.8-96.3)	85.4 (76.2-85.4)	85.5 (68.9-95.3)	n.s.
"Normal" $FEV_1 \geq 80\%$, n, %	67 (34%)	25 (35.7%)	42 (33.6%)	n.s.
$MMEF_{25/75}$% pred. (median, IQR)	52.3 (39.3-68.7)	53.6 (41.1-71.0)	50.0 (35.5-65.7)	n.s.
BDR (ΔFEV_1% init. ≥12%), n, %	112 (55.4%)	44 (56.4%)	68 (54.8%)	n.s.
BDR (ΔFEV_1% init. ≥11%), n, %	117 (57.9%)	46 (59.0%)	71 (57.3%)	n.s.

BDR (ΔFEV_1% init. ≥10%), n, %	123 (60.9%)	50 (64.1%)	73 (58.9%)	n.s.
BDR (ΔFEV_1% init. ≥9%), n, %	132 (65.3%)	53 (67.9%)	79 (63.7%)	n.s.
Uncontrolled ACQ7≥1.5, %	54 (49.5%)	21 (53.8%)	33 (41.7%)	n.s.
Partially controlled ACQ7 (0.75-1.5), %	16 (14.7%)	6 (15.4%)	10 (14.3%)	n.s.
Controlled ACQ7<0,75, %	39 (35.8%)	12 (30.8%)	27 (38.6%)	n.s.
Elevated total IgE n, %	50 (61.7%)	18 (69.2%)	32 (58.2%)	n.s.
Specific IgE≥1 EAST class n, %	90 (82.6%)	34 (87.2%)	56 (80%)	n.s.

Abbreviations - ACQ, IQR - interquartile range, FEV_1 - forced expiratory volume in 1 second, $MMEF_{25/75}$ - maximum mid-expiratory flow during the mid (25-75%) portion of the FVC, BDRT - bronchodilator response. *comparison between boys and girls; #history data for the last 12 months.

Table S2. Correlation analysis between the study groups of asthmatic children regarding their asthma severity assessed by: GINA control treatment step, initial spirometry result (baseline FEV_1), and severity of the symptoms between exacerbations (GINA symptom score and ACQ6 score) (SABA - short acting beta-agonist)

Asthma severity	GINA step "Intermittent asthma" SABA N (%)	GINA step "Persisting Asthma"		
		Step 1 and 2 Mild N (%)	Step 2 and 3 Moderate N (%)	Step 4 and 5 Severe N (%)
Baseline spirometry				
FEV_1>100%	19 (17.0%)	17 (30.9%)	5 (23.8%)	0 (0%)
FEV_1>80%	50 (44.6%)	23 (41.8%)	10 (47.6%)	2 (66.7%)
FEV_1 60-80%	31 (27.7%)	15 (27.3%)	3 (14.3%)	1 (33.3%)
FEV_1<60%	12 (10.7%)	0 (0%)	3 (14.3%)	0 (0%)
Symptoms between exacerbations - GINA symptom score				
No symptoms	18 (28.6%)	8 (24.2%)	2 (16.7%)	1 (100%)
1-2 symptoms	12 (19.0%)	11 (33.3%)	3 (25.0%)	0 (0%)
3-4 symptoms	20 (31.7%)	8 (24.2%)	5 (41.7%)	0 (0%)
5-6 symptoms	13 (20.6%)	6 (18.2%)	2 (16.7%)	0 (0%)
Symptoms between exacerbations - ACQ6				
Well controlled (<0.75)	21 (33.3%)	14 (42.4%)	3 (25.0%)	1 (100%)
Partial controlled (0.75-1.5)	9 (14.3%)	5 (15.2%)	2 (16.7%)	0 (0%)
Non controlled	33 (52.4%)	14 (42.4%)	7 (58.3%)	0 (0%)
*	n.s.	n.s.	n.s.	n.s.

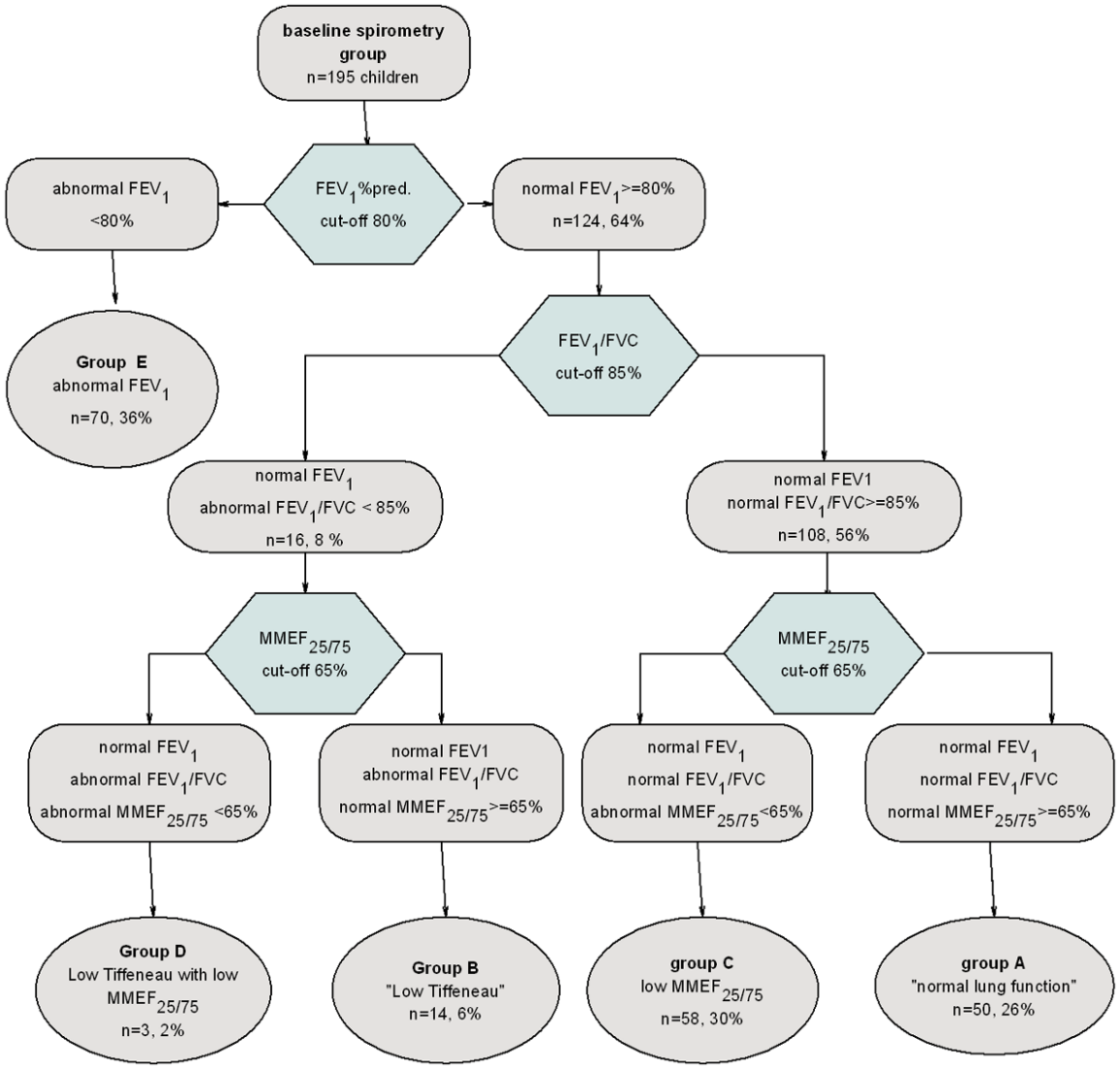

Figure S2. Study subgroups determination.

Table S3. Comparison between children with normal spirometry with/without SAO and those with reduced baseline FEV_1

Parameter	SAO FEV_1≥80% $MMEF_{25/75}$<65%	Normal spirometry FEV_1≥80% $MMEF_{25/75}$≥65%	p*	Abnormal baseline spirometry FEV_1<80%	p#
Controlled[1]	7 (22.6%)	21 (31.3%)	n.s.	6 (23.1%)	n.s.
Partly controlled[1]	3 (9.7%)	10 (14.9%)		4 (15.4%)	
Uncontrolled[1]	21 (67.7%)	36 (53.7%)		16 (61.5%)	
Hospitalizations ≥1	24 (42.9%)	53 (47.7%)	n.s.	24 (44.4%)	n.s.
Exacerbations ≥1	42 (76.4%)	87 (79.1%)	n.s.	40 (74.1%)	n.s.
Exercise induced exacerbation (n, %)	16 (29.1%)	28 (25.2%)	n.s.	10 (19.6%)	n.s.
Physical activity limitation (n, %)	14 (25.5%)	29 (26.4%)	n.s.	16 (32.0%)	n.s.
Allergen induced exacerbation (n, %)	12 (21.8%)	22 (19.8%)	n.s.	10 (20.0%)	n.s.
School absence, days (>5 days)	21 (42.9%)	49 (50.5%)	n.s.	36 (72.0%)	n.s.
Allergic rhinitis	39 (56.5%)	71 (56.3%)	n.s.	33 (50.8%)	n.s.

BMI	18.37 (16.4-21.8)	18.08 (16.0-22.2)	n.s.	18.40 (16.2-23.2)	n.s.
Allergic sensitization[2]	28 (90.3%)	61 (91.0%)	n.s.	20 (76.9%)	n.s.
Allergic sensitization[3]	1 (3.2%)	2 (2.9%)	NA	2 (7.7%)	NA
House dust[3]	12 (24.2%)	31 (16.9%)	0.037	12 (17.9%)	n.s.
Birch[3]			n.s.		n.s.
Pets[3]	10 (32.3%)	23 (65.7%)	n.s.	9 (34.6%)	n.s.
Molds[3]	12 (38.7%)	27 (40.3%)	n.s.	11 (42.3%)	n.s.
Tree/grass pollens[3]	13 (41.9%)	30 (44.8%)	n.s.	13 (50.0%)	n.s.

*comparison between SAO group and children with normal baseline spirometry; #comparison between SAO group and children with abnormal baseline spirometry; [1]ACQ6; [2]specific IgE>1 EAST class; [3]specific IgE>3 EAST class; NA - non-applicable.

Table S4. Distribution of asthmatic children with peripheral obstruction depending on baseline FEV_1

$MMEF_{25/75}$ value	Decreased function (FEV_1<80%)		"Normal" function (FEV_1≥80%)		p
	N	Percent	N	Percent	
<65%	66	98.5%	71	55.9%	<0.0001
≥65%	1	1.5%	56	44.1%	

Table S5. Binary logistic regression analysis for the relationship between the presence of peripheral obstruction ($MMEF_{25-75}$<65%) and the likelihood of occurrence of a risk domain

Index	Level of significance, p	HR	95% C.I. Lower limit	95% C.I. Upper limit
$MMEF_{25/75}$ (< and ≥65%)	0.023	2.270	1.120	4.603
Constant	0.022	1.491		

Table S6. Comparison of median $MMEF_{25-75}$/FVC among children with asthma and healthy controls

baseline $MMEF_{25-75}$/FVC index (Forced expiratory decay)				p
Asthma group	Median		0.615	<0.0001
	IQR	25	0.476	
		50	0.615	
		75	0.757	
Healthy control group	Median		1.052	
	IQR	25	0.935	
		50	1.052	
		75	1.305	

Table S7. Pre- and post-$MMEF_{25-75}$/ FVC (flow-volume loop shape) in children with asthma

$MMEF_{25-75}$/FVC index		Pre-$MMEF_{25-75}$/FVC	Post-$MMEF_{25-75}$/FVC	p
N	Valid	192	190	<0.0001
	Missed	19	21	
Median		0.615	0.748	
IQR	25	0.476	0.570	
	50	0.615	0.748	
	75	0.757	0.908	